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(54) Title: THERMOPROTECTED MICROPARTICLE COMPOSITIONS AND PROCESS FOR TERMINAL STEAM STERILIZATION THEREOF

(57) Abstract

Compositions of submicron– to micron–sized particles of water–insoluble biologically active substances that are stabilized by thermoprotecting agents, can be terminally steam sterilized without any significant increase of mean particle size. These compositions display markedly reduced heat–induced coagulation, flocculation, or particle size growth during the terminal steam sterilization process.

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THERMOPROTECTED MICROPARTICLE COMPOSITIONS AND PROCESS FOR TERMINAL STEAM STERILIZATION THEREOF

BACKGROUND

Several compositions of micro- and nano-particle suspensions of water-insoluble or poorly water-soluble biologically active substances such as pharmaceutical agents, and methods to prepare such suspensions have been described in patent literature. These compositions use surfactant molecules as surface modifiers that associate on the surface of the micro- or nano-particles and inhibit the growth of their size. Such surface stabilized microparticles may be administered to elicit their pharmaceutical advantage by injectable or oral or other routes of administration.

Drug delivery systems utilizing microparticulate suspensions have been described in literature (D. H. Haynes, "Phospholipid-coated Microcrystals: Injectable Formulations of Water-Insoluble Drugs." US Patents 5,091,187 and 5,091,188). These suspensions are believed to be the first applications of the surface modified microparticulate aqueous suspension containing particles made up of a core of pure drug substances and stabilized with natural or synthetic bipolar lipids including phospholipids and cholesterol. Subsequently, similar delivery systems exploiting these principles have been described (G.G. Liversidge et al., "Surface Modified Drug Nanoparticles." US Patent 5,145,684 K. J. Illig and P. Sarpotdar, "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability." US Patent 5,340,564 H. William Bosch et al., "Process for Preparing Therapeutic Compositions Containing Nanoparticles." US Patent 5,510,118) emphasizing the usefulness of the drug delivery approach utilizing particulate aqueous suspensions.

Sterilization of the submicron- to micron-sized particle suspension of the pharmaceutical agent is necessary for their parenteral administration. The preferred method of sterilization of pharmaceutical parenteral agents is terminal sterilization by autoclaving. It has been found that many surface modified submicron- to micron-sized particle suspensions undergo particle size growth during autoclaving. This is attributed to the release of the surfactant molecules from the small particle surface and its subsequent coagulation at autoclaving temperatures. The small particles that are devoid of the surfactants become

unstabilized and undergo particle size growth by various mechanisms. The temperature at which such coagulation of surfactant molecules occur is known as the cloud point of that surfactant. It is believed that addition of cloud point modifiers, which are merely other surfactants, raises the cloud point of the primary surfactant and thereby maintaining the surface modifier coating on the nanoparticles during autoclaving. The cloud point modifier molecules described in majority of the published literature (US Patent 5,298,262 US Patent 5,336,507, and US Patent 5,340,564) are ionic surfactants, including charged phospholipids.

Successful terminal steam sterilization of phospholipid-stabilized emulsions and phospholipid-liposomes have been reported in literature [1-4]. However, examples of successful terminal steam sterilization of micron or submicron size particle suspensions of water insoluble or poorly soluble drugs, that contain only phospholipids as the surface modifier, have not been reported prior to the findings reported in the present invention.

DESCRIPTION OF THE INVENTION

Surprisingly, it was found that selected compositions of submicron- to micron-sized particulate suspension of water-insoluble or poorly water-soluble pharmaceutical agents containing a pharmaceutically acceptable water soluble polyhydroxy compound could be autoclaved without any marked increase of mean particle size.

Yet another surprising finding was that such compositions withstood the stresses that are usually known to promote particle size growth or flocculation or agglomeration. For instance, without any significant increase in particle size, the steam sterilized compositions could be shaken for several days, could withstand the stress due to cyclical storage at 40 and 5°C, repeated freezing and thawing, or severe sedimentation forces.

It was a further surprising finding that these compositions could be successfully lyophilized before or after steam sterilization. In addition, the lyophilized preparations could be reconstituted by addition of water to make an aqueous suspension having qualities similar to the original suspension.

These compositions did not use any surfactants that would require cloud point modifying molecules for protection against coagulation, flocculation, crystal growth, or particle size growth during the terminal steam sterilization process. The steam sterilizable formulations described in the present invention differ from those known in the art by the absence of surfactants which have a tendency to coagulate on steam sterilization, e.g.,

polyvinylpyrrolidone, and presence of "thermoprotecting excipients as well as other thermoprotecting conditions" as described below.

The present invention focuses on how the growth of particles can be prevented during and after terminal steam sterilization of micron and sub-micron sized particles of water insoluble or poorly soluble pharmaceutical agents due to certain types of agents defined here as "thermoprotecting agents", and selected processing conditions defined here as "thermoprotecting conditions".

The "thermoprotecting agents" and "thermoprotecting conditions" are characterized by their ability to restrict the increase in volume weighted mean diameter of the particulate suspension during and after terminal steam sterilization to a limit that the steam sterilized suspension can be injected by intravenous or other parenteral routes of administration without compromising the safety of the subject. A volume weighted mean diameter of up to about 3 μm may is considered safe for intravenous injection. However, such suspension should not contain more than 3000 particles of 10μm or greater size and not more than 300 particles of 25μm or greater size according to the USP particulate test criterion. We have thus defined the term "successful steam sterilization" as a process with which one can prepare formulations which does not contain particles of above specified diameter limits or preferably the volume weighted mean particle diameter of the suspension does not increase after steam sterilization by more than about two-times.

While the surface modifiers possibly adsorb to the freshly made surfaces of drug particles during the process of particle size reduction, and (a) convert lipophilic drug surface to hydrophilic surface that has increased stability, and (b) possibly modify the surface charge of the drug particle surfaces, the thermoprotecting agent and thermoprotecting conditions described herein help maintain the particle size distribution of the suspension during and after the terminal steam sterilization conditions.

Examples of suitable thermoprotecting agents include one or a combination of pharmaceutically acceptable water soluble polyhydroxy compounds that also act as tonicity modifiers, such as dextrose, sucrose, mannitol, sorbitol, dextran, trehalose, lactose. A detailed description of these agents may be found in *Remington's Pharmaceutical Sciences*, 18th Edition, 1990, Mack Publishing Co., PA; and *Theory and Practice of Industrial Pharmacy*, Lachman *et al.*, 1986.

Suitable thermoprotecting conditions include absence of high ionic strength, particularly absence of high concentration of hydrogen or hydroxyl ions. Some other suitable thermoprotecting conditions include absence of agents such as polyethylene glycols, polyvinyl alcohol, polyvinylpyrrolidone, which themselves have a natural tendency to coagulate at high temperatures.

Without wishing to limit this invention to any particular theory, it is thought that some of the functions of the combination of surface active or non-surface active thermoprotecting agents and thermoprotecting conditions as they relate to this invention are:

- To suppress the process of Ostwald Ripening during the cooling cycle of the terminal steam sterilization and therefore maintain the particle size, increase the storage stability, minimize sedimentation, and decrease the particle growth while lyophilization and reconstitution.
- To enhance the association of surface modifier and the drug particles such that the protecting environment around the particles is maintained over a wide range of temperature and pressure as is prevalent during the terminal steam sterilization process.
- To increase the interface compatibility between water-insoluble drug particles and the liquid.
- To aid in orienting the surface modifiers' hydrophilic portion preferentially into the aqueous phase while the lipophilic portion remains strongly adsorbed to the surface of the water-insoluble drug particle as well as to enhance the stability of such orientation.

The process that can be used to produce these stable sub-micron and micron size particles include mixing the drug with phospholipid, other surfactants, thermoprotecting agents, and other ingredients followed by sonication, milling, homogenization, microfluidization, and antisolvent and solvent precipitation, spray drying of the solution in compressed normal or supercritical solvents.

Examples of some preferred water-insoluble drugs include antifungal agents, immunosuppressive and immunoactive agents, antiviral agents, antineoplastic agents, analgesic and antiinflammatory agents, antibiotics, antiepileptics, anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, antagonists, neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergic and, antiarrhythmics, antihypertensive

agents, antineoplastic agents, hormones, and nutrients. A detailed description of these drugs may be found in *Remington's Pharmaceutical Sciences*, 18th Edition, 1990, Mack Publishing Co., PA.

EXAMPLES

Example 1

Table I summarizes some of the example compositions and observations. In Table I is displayed the amounts of drug substance (itraconazole), egg-phospholipid (surface modifier), and tonicity agents (various polyhydroxy compounds) used in making those preparations. These compositions do not require addition of so-called cloud point modifying agents to prevent egg-phospholipid separation and coagulation. The attributes of the suspensions made before and after terminal steam sterilization is also included in this table.

These preparations have been made by mixing the ingredients with appropriate amount of water, adjusting the pH with the required quantities of aqueous sodium hydroxide, and then subjecting the dispersion to high pressure homogenization or high pressure microfluidization at pressures in the range of 10000 psi 25000 psi. During the homogenization or microfluidization process the process fluid was cooled to maintain a temperature between 5-35°C. The finished product was filled in 5 or 10 mL borosilicate USP Type I glass vials. These vials were sealed under nitrogen atmosphere and subjected to terminal steam sterilization at 121°C for 15 to 30 minutes.

Successfully terminally steam sterilized preparations of itraconazole, experiments 1-A through 1-G, are displayed in Table I. By the term "successfully terminally steam sterilized preparations" it is understood in this example that the volume weighted mean particle diameter of the suspension did not increase after steam sterilization by more than two-times. To demonstrate this, Table-I shows the ratio of post-autoclaving mean particle size to that before sterilization, which are within 1.04 to 1.18. The volume-weighted diameters of these suspensions have been determined with a Malvern Mastersizer Microplus, which utilizes a method based on the diffraction of light by the particulate suspension.

Formulations 1-A to 1-G described in Table-I are examples of successful steam sterilized products without any significant increase in particle size. Volume weighted mean diameters of the suspensions after terminal steam sterilization for the said formulations did not increase by more than a factor of two.

TABLE I: Examples of terminally steam sterilized Microparticle-Itraconazole suspensions and their pre- and post-sterilization attributes.

Formulation Number	1-A	1-B	1-C	1-D	1-E	1-F	1-G
Drug Amount, %	2	5	10	9	9	9	10
Lipoid E80, %	0.5	1.1	3.5	2.7	2.7	2.7	2.0
Other Additive*	TRE	TRE	TRE	DE38	DE77	LAC	MAN
Other Additive, %	12	12	13	10	10	10	5.5
Water	qs 100%	qs 100%	qs 100%	qs 100%	qs 100%	qs 100%	qs 100%
Drug:Lipid Ratio	4:1	4.5:1	2.86:1	3.33:1	3.33:1	3.33:1	5:1
Pre-Sterilization Particle Size, μm	1.07	1.01	0.9	1.30	1.30	1.31	0.75
Post-Sterilization Particle Size, μm	1.16	1.16	1.03	1.53	1.5	1.45	1.27
Post- to Pre-Sterilization Particle Size Ratio	1.08	1.14	1.14	1.18	1.15	1.11	1.69

* Symbols and sources of chemicals: Itraconazol (Wyckoff Chemical Co.); TRE = Trehalose (Pfanstiehl, Waukegan, IL); DE38 = Dextran-average molecular weight = 38,100 (Sigma, St. Louis, MO); DE77 = Dextran-average molecular weight = 77,000 (Sigma, St. Louis, MO); LAC = Lactose (BDH Inc., Toronto, Canada); MAN = Mannitol (J. T. Baker, Phillipsburg, NJ); GLY = glycerin.

Example 2

In Table II are presented the results of some negative control experiments. As a control experiment, an itraconazole formulation (2-A) without any thermoprotectant and surface modifier addition was attempted. The solid drug could not be dispersed in water. Major portion of the drug remained floating on the surface of water. Therefore, it could not be homogenized. It was found that addition of a surfactant was necessary that also acted as a wetting agent. This formulation could not be made possible without any surface modifier. Therefore, steam sterilization and particle size determinations were not attempted.

The formulation 2-B to 2-E were prepared by the method described in Example 1.

TABLE II: Examples of terminally steam sterilized Microparticle-Itraconazole suspensions and their pre- and post-sterilization attributes.

Formulation Number	2-A	2-B	2-C	2-D	2-E
Drug: Itraconazole	10%	10%	2.5%	8.1%	8.1%
Lipoid E80	0%	10%	10%	2.4%	2.4%
Other Additives[1]	0%	MAN: 5.5%	GLY: 2.5%	TRE: 12% MRJ: 2.0%	TRE: 12% PF68: 2.0%
Water	qs 100%	qs 100%	qs 100%	qs 100%	qs 100%
Drug:Lipid Ratio	NA	1:1	0.25:1	3.4:1	3.4:1
Pre-Sterilization Particle Size, μm	ND[2]	0.59	ND[4]	0.86	0.86
Post-Sterilization Particle Size, μm	ND[2]	ND[3]	ND[4]	7.84	4.22
Post- to Pre-Sterilization Particle Size Ratio	ND[2]	ND[3]	ND[4]	9.1	4.9

Notes:

[1] Symbols and sources of chemicals: Itraconazol (Wyckoff Chemical Co.); Lipoid E80 (Lipoid gmbH); TRE = Trehalose (Pfanstiehl, Waukegan, IL); MRJ = Myrj52S (ICI Surfactants); PF68 = Pluronic F68 (BASF); MAN = Mannitol (J. T. Baker, Phillipsburg, NJ); GLY = glycerin.

[2] The solid drug could not be dispersed in water, therefore, it could not be homogenized. It was found that addition of a surfactant was necessary that also acted as a wetting agent. This formulation could not be made possible without any surface modifier. Therefore, steam sterilization and particle size determinations were not attempted.

[3] Formulation 2-B demonstrated flocculation or aggregation and significant quantity of scum formation on the surface of the autoclaved material which dispersed slowly on vigorous agitation.

[4] Particle size of the formulation 2-C, consisting of 2.5% glycerol as the tonicity modifier, showed highly unstable particle size and therefore terminal steam sterilization was not performed.

The formulation 2-B demonstrated flocculation or aggregation and significant quantity of scum formation on the surface of the autoclaved material, which dispersed slowly on vigorous agitation. It was thought that the flocculation or creaming on steam sterilization of formulation 2-B originated from an excessive amount of phospholipid. This formulation has a

1:1 ratio of drug to Lipoid E80, *i.e.*, 10% w/w each. It is believed that excessive amount of phospholipid resulted in some sort of cross-linked structure during steam sterilization that induced flocculation and creaming.

Additionally, in presence of a large excess of the surfactants during the steam sterilization conditions the particle size growth may occur due to solubilization of the drug in the microstructures of surfactant molecules followed by recrystallization upon cooling. Such microstructures include minute quantities of micelles or liposomes in equilibrium with other structures formed with the surfactant molecules. The fraction of these microstructures would increase with increasing quantities of the surfactants. It was thus recognized that maintaining a proper amount of the surface modifier in the formulation was important in order to avoid the particle size growth upon terminal steam sterilization.

In general, terminal steam sterilization of the microparticle formulations was found to be successful by reducing the phospholipid to a minimum quantity (*e.g.*, from ~10% w/w to about 2-5% w/w) that could allow an effective coating of the phospholipid on the drug-microparticle while avoiding the undesirable phospholipid structures considered to be responsible for large size cross-linked structures on steam sterilization. A drug to phospholipid ratio above about 3:1 seemed to give good result (formulations 1-A to 1-G of Example 1). When the drug to phospholipid ratio was brought down, *e.g.*, from 5:1 in formulation 1-G (Example 1), to 1:1 in formulation 2-B, extensive flocculation or aggregation and significant quantity of scum formation on the surface of the autoclaved material was observed.

Particle size of the formulation 2-C, consisting of 2.5% glycerol as the tonicity modifier, was unstable and therefore terminal steam sterilization was not performed. This formulation had a large quantity of phospholipid compared to the drug, giving a low drug to phospholipid ratio of 0.25:1. In addition, this formulation employed 2.5% w/w glycerin as the tonicity modifier. It is believed that the unfavorable drug: phospholipid ratio and/or use of glycerin as the tonicity modifier caused the observed increase in the particle size of the formulation even without the heat stress of terminal steam sterilization.

Formulations 2-D and 2-E represent the effect of addition of certain commonly used surfactants. Surfactant Myrj-52S (polyethyleneglycol-40 sterate) was present at 2.0% in formulation 2-D in addition to 2.4% Lipoid E80 and 8.1% itraconazole. Similarly, surfactant Pluronic F68 (a Poloxamer) was present at 2.0% in formulation 2-E in addition to 2.4% Lipoid

E80 and 8.1% itraconazole. Although the mean particle size of the preautoclaved suspension of both formulations 2-D and 2-E remained 0.86μm, upon steam sterilization it increased tremendously to 7.84 and 4.22 μm, respectively. Both the formulations became highly viscous after steam sterilization. The formulations 2-D and 2-E display the post- to pre-sterilization particle size ratios of 9.1 and 4.9 respectively. This experiment demonstrates that addition of certain surfactants to Lipiod E80 containing Microparticle formulations results in a large growth of particle size.

Example 3

Preparation "C" (Microparticle-Itraconazole (10%)) of the example 1 was used for this experiment. Approximately 5 g of the preparation was placed in a vial and sealed under nitrogen. Freeze/thaw stress was given as follows. The vial contents were frozen by storing in a freezer (approximately –20°C) for at least 6 hours. The frozen sample was then thawed by placing the vial at room temperature for 0.5-1 hour. Particle size distribution of the thawed sample was measured by the method mentioned above. Appearance of the thawed sample was recorded. The vial was then again sealed under nitrogen for the next cycle of this experiment. The results of this experiment are summarized in Table III. The formulation has displayed a very good particle size stability upon the destabilizing stress of freeze/thaw conditions.

Example 4

A thermal cycling stress was given to the preparation "1-C" of example 1 by storing the formulation for approximately 24 hours in a refrigerator at about 4°C and then in an incubator at about 40°C for approximately 24 hours. The particle size was measured and appearance noted at the end of each cycle. This cycle was repeated. The results are given below in Table IV. The results indicate a very good stability of the particle size and appearance of the formulation on thermal cycling stress. The formulation remained stable for 4 cycles, after which the study was terminated.

Table III: **Particle size stability of Microparticle-Itraconazole (10%) on freeze/thaw stress.**

Cycle #	Volume Weighted Particle Size, μm			Appearance
	Mean	90 Percentile	99.9 Percentile	
0	1.04	1.60	2.52	Homogeneous White Suspension
1	1.04	1.60	2.52	
2	1.01	1.53	2.47	
3	1.01	1.52	2.44	
4	1.05	1.61	2.53	
5	1.02	1.52	2.44	
6	1.01	1.50	2.38	
7	1.02	1.54	2.41	
8	1.03	1.55	2.42	
9	1.02	1.53	2.44	
10	1.03	1.57	2.47	

Table IV: **Particle size stability of Microparticle-Itraconazole (10%) on thermal cycling (4-40°C) stress.**

Cycle #	Volume Weighted Particle Size Distribution (μm)			Appearance
	Mean	**90 Percentile**	**99.9 Percentile**	
0	1.04	1.60	2.52	Homogeneous White Suspension
1	1.01	1.52	2.45	
2	1.02	1.56	2.47	
3	1.02	1.57	2.50	
4	1.03	1.59	2.76	

Example 5

Good stability on shaking stress has been also demonstrated (see Table V). The steam-sterilized formulation of example "1-C" was tested. Shaking stress was given as follows. The vial containing the formulation was placed horizontally on an orbital shaker and shaken at approximately 100 rpm. The vial was removed from the shaker daily for observation of the appearance. Particle size was measured every alternate day. The volume weighted mean particle size and its 90 as well as 99.9 percentile did not change significantly on shaking for 7 days. The study was terminated after 7 days.

Table V: Particle size stability of Microparticle-Itraconazole (10%) on shaking stress

Shaking Stress Time Point	Volume Weighted Particle Size (μm)			
	Mean	90 Percentile	99.9 Percentile	Appearance
Day 0	1.04	1.60	2.52	Homogeneous White Suspension
Day 3	1.05	1.64	2.83	
Day 5	1.10	1.77	3.28	
Day 7	1.06	1.68	2.83	

Example 6

The long-term sedimentation behavior was assessed by a centrifugation experiment and the formulation quality was determined by particle sizing (Table VI). Preparation "1-C" of example 1 was tested. The formulation could not be sedimented by centrifuging for approximately 20 min at 3000-rpm. Significant sedimentation was observed by increasing the centrifugation speed to approximately 5000 and 6000 rpm for another 20 min., however this sediment was resuspendable with some difficulty upon shaking. Resuspendibility was assessed as: Easy: Sedimented suspension became visually homogeneous on shaking gently by hand. Moderate: Sedimented suspension became visually homogeneous on vigorous hand shaking. Difficult: Vortexing required for the sedimented suspension to make visually homogeneous.

There was no increase in particle size upon such sedimentation. In addition, agglomeration or flocculation was not observed in optical microscopy. Average particle size by the optical microscopy agreed with that by Malvern Mastersizer.

Table VI: Stability of Microparticle-Itraconazole (10%) on sedimentation stress

Centrifuging Condition		Sedimentation	Resuspendibility	Volume Weighted Particle Size (μm)		
Speed (rpm)	Duration (min)			Mean	90 Percentile	99.9 Percentile
Before Centrifugation		None	NA*	1.05	1.58	2.48
1000	5	None	NA	ND*	ND	ND
1500	10	None	NA	ND	ND	ND
2000	15	Little	Easy	1.02	1.51	2.39
3000	15	Little	Moderate	0.99	1.47	2.20
5000	15	Significant	Difficult	0.97	1.43	2.19
6000	15	Significant	Difficult	0.99	1.46	2.17

*NA = Not Applicable; ND = Not Determined.

Example 7

Preparation "1-C" (Microparticle-Itraconazole (10%)) of the Example 1 was used for this experiment. Approximately 5g of the unautoclaved product was placed in a glass vial and lyophilized. The vials that were terminally steam sterilized were also lyophilized. The lyophilized material was an off-white cake. The lyophilized cake was easily reconstituted with water by 4-5 gentle inversions of the vial into a homogenous white suspension. The appearance and particle size of the original suspension and that of lyophilized and reconstituted preparation is presented in Table VII. Both the unautoclaved and autoclaved formulations display good particle size stability upon lyophilization and reconstitution.

Example 8

The formulations and their attributes of this example are given in Table VIII. These formulations were prepared by the methods of Example 1. In the microparticle-cyclosporine formulation 8-A, polyhydroxy compound acting as thermoprotectant or tonicity modifier was not added into the premix. The particle size reduction profile was found to be very inefficient. The volume weighted mean particle diameter of the suspension was about 4 micrometers at the end of homogenization. This suspension was steam sterilized at 121°C for 15 minutes that resulted in a heavy coagulated mass of the solid particles of several millimeters. Almost all of the drug substance was seen sedimented leaving behind a clear supernatant.

Table VII: Particle size stability upon lyophilization and reconstitution of a Microparticle-Itraconazole (10%) Suspension

Formulation Condition	Appearance	Volume Weighted Particle Size (μm)		
		Mean	90 Percentile	99.9 Percentile
Unsterilized Suspension Before Lyophilization	Homogeneous White Suspension	0.9	1.31	2.08
Unsterilized Lyophilized and Reconstituted Suspension	Homogeneous White Suspension	1.00	1.60	2.56
Sterilized Suspension Before Lyophilization	Homogeneous White Suspension	1.03	1.59	2.51
Sterilized Lyophilized and Reconstituted Suspension	Homogeneous White Suspension	1.10	1.71	2.51

Table VIII: More examples of terminally steam sterilized microparticle formulations.

Formulation Number	8-A	8-B
Drug	Cyclosporine	Cyclosporine
Drug Amount, %	10	10
Trehalose, %	None	12
Lipoid E80, %	3.0	3.0
Pre-Sterilization Particle Size, μm	~ 4	0.72
Post-Sterilization Particle Size, μm	Large Particles by Visual Inspection	1.03
Ratio of Post- and Pre-Sterilization Particle Sizes	Much greater than 2	1.43

Premix of formulation 8-B contained trehalose in addition to the components of example 8-A. The homogenization process of this formulation was interrupted in the midway by allowing to stand overnight under nitrogen atmosphere at ambient temperature. The homogenization was completed the next day. Efficient particle size reduction to a volume weighted mean diameter of 0.72 micrometers was observed. In addition, this formulation could be successfully steam sterilized at 121°C for 15 minutes with an acceptable increase in the particle size to approximately 1.03, an increase by a factor of only 1.43. It is believed that the presence of the polyhydroxy compound, trehalose, allowed the efficient particle size reduction. The formulation could withstand the heat stress of autoclaving without a large increase in the

particle size.

Example 9

Some example formulations containing Alfaxalone and their pre and post steam sterilization attributes are shown in Table IX. These formulations were prepared by the methods of **Example 1.**

Table IX: Examples of terminally steam sterilized Microparticle-Alfaxalone formulations.

Formulation Number	9-A	9-B	9-C
Drug Amount, %	3.0	3.0	3.0
Lipoid E80, %	2.0	2.0	1.0
DSPC, %*	1.0	1.0	0.5
DMPG, %**	0.2	0.2	0.1
Dextran, %	20	---	20
Sodium Chloride, M	---	---	---
Water	qs 100%	qs 100%	qs 100%
Pre-Sterilization Mean Particle Size, μm	1.38	1.38	1.42
Post-Sterilization Mean Particle Size, μm	2.95	5.24	2.71
Ratio of Post- and Pre-Sterilization Mean Particle Sizes	2.1	3.8	1.9

* DSPC = disteroylphosphatidyl choline

** DMPG = dimyristoylphosphatidyl glycerol

Formulation, 9-A, which has a combination of phospholipids (Lipoid E80, DSPC and DMPG) and dextran as the thermoprotectant, demonstrates about 2-fold increase in particle size upon steam sterilization by heating at 121°C for 15 min. On the other hand, formulation 9-B, which has composition similar to that of 9-A except the absence of dextran, shows a much higher mean particle size (5.24μm) and the ratio of post- and pre-sterilization mean particle sizes of 3.8. Thus presence of dextran in formulation 9-A has improved the particle size stabilization over that of formulation 9-B. Formulation 9-C is very similar to the formulation 9-A except slightly different amounts of surface modifiers. In this formulation also the particle size increase has been limited to about a factor of two. It has a mean particle size of 2.71μm

and the ratio of post- and pre-sterilization mean particle sizes of only 1.9.

In addition to the example compositions mentioned above, the formulations of this invention may additionally contain suitable amount of pH buffering salts and pH adjusting agents such as sodium hydroxide and/or pharmaceutically acceptable acids. It is known to those skilled in the art of handling the phospholipids that at pH lower than 5 and higher than 9 the phospholipid molecules undergo extensive hydrolysis. Therefore, the pH of the suspension was usually adjusted to within this range prior to homogenization, and if necessary readjusted prior to steam sterilization.

While the invention and the examples have been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not to be limited to the disclosed embodiment, but on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the following claims.

REFERENCES

1. "Cyclosporin emulsions", Parikh, Indu; and Mishra, Awadhesh; US # 5660858, 1997.

2. "Composition for enhancing the administration of pharmacologically active agents", Wretlind, Karl A. J.; Ljungberg, Stellan; Hakansson, Ivan; Ajaxon, Bengt M.; USRE # 032393, 1987.

3. "Sterilization of liposomes by heat treatment" by Zuidam, Nicolaas J.; Lee, Stephan S.L.; and Crommelin, Daan J.A.; *Pharmaceutical Research* **10**:1592-1596, 1993.

4. "Liposomes" Klaveness, Jo; Berg, Arne; Jacobsen, Trond Vegard; Rongved, Pal; Ege, Thorfinn; Kikuchi, Hiroshi; Yachi, Kiyoto; US5676928, 1997.

WHAT IS CLAIMED IS

1. An aqueous suspension composition of water insoluble or poorly soluble biologically active substance together with at least one surface modifier and a pharmaceutically acceptable, water soluble polyhdroxy thermoprotecting agent, the ratio of active substance to surface modifier and thermoprotecting agent selected to provide particle size stability during and after terminal steam sterilization, provided that the particle size is not more than an about two-fold increase in the volume weighted mean particle size of the particulate aqueous suspension subsequent to terminal steam sterilization.

2. An aqueous suspension composition of water insoluble or poorly soluble biologically active substance together with at least one surface modifier and a pharmaceutically acceptable, water soluble polyhdroxy thermoprotecting agent, the ratio of active substance to surface modifier and thermoprotecting agent selected to provide particle size stability during and after terminal steam sterilization, provided that the particle size is not more than an about two-fold increase in the volume weighted mean particle size of the particulate aqueous suspension subsequent to terminal steam sterilization, wherein the composition is substantially completely devoid of surfactants that require elevation of their cloud point temperature by addition of a cloud point modifier for further stabilization and substantially devoid of surfactant additives which cause destabilization of the formulation.

3. The composition of claim 1 wherein the pH of the suspension before terminal steam sterilization is between about 5 to about 9 provided the pH value prior to terminal steam sterilization is selected such that the chemical stability of the suspension components is maintained during and after the terminal steam sterilization step.

4. The composition of claim 1 wherein the composition also includes an amount of non-surfactant additives such that the composition attains a suitable osmotic pressure for safe parenteral administration.

5. The composition of claim 1 wherein the composition also includes an amount of non-surfactant additive such that, on diluting the formulation with pharmaceutically acceptable diluent suitable for parenteral administration to a pharmaceutically acceptable concentration for parenteral administration, a suitable osmotic pressure of the diluted suspension results.

6. The composition of claim 1 wherein the thermoprotecting agent is a pharmaceutically acceptable water soluble polyhydroxy compound selected from the group consisting of trehalose, lactose, dextrose, sorbitol, dextran, trehalose and mannitol.

7. The composition of claim 1 wherein one or more of the surface modifiers are natural phospholipids or synthetic phospholipids.

8. The composition of claim 7 wherein the surfacemodifier is an egg phospholipid or soy phospholipid.

9. The composition of claim 1 wherein the amount of the surface modifier provides drug to surface modifier ratio of up to 5:1.

10. The composition of claim 1 wherein the amount of surface modifier is in the range from about 0.2% w/w to about 5.0% w/w.

11. The composition of claim 1 wherein the composition also contains pharmaceutical excipients for ophthalmic, peroral, or transdermal administration of the water insoluble or poorly soluble active drug substance.

12. The composition of claim 1 wherein the active substance is an antifungal agent

13. The composition of claim 12 wherein the active substance is itraconazole.

14. The composition of claim 1 wherein the active substance is an immuno-suppressive drug.

15. The composition of claim 14 wherein the active substance is a cyclosporin

16. The composition of claim 1 wherein the active drug is a sterol

17. The composition of claim 16 wherein the active drug is a alfaxalone

18. A lyophilized or spray dried powder prepared from the composition of claim 1.

19. A composition according to claim 1 wherein the water-insoluble or poorly water-soluble active drug substance is at a concentration suitable for either immediate release or sustained release delivery of the drug by parenteral administration.

20. The composition of claim 19 wherein the parenteral administration is intramuscular, or subcutaneous administration.

INTERNATIONAL SEARCH REPORT

Inter . inal Application No
PCT/US 99/11888

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/14 A61K9/51 A61K9/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 44 40 337 A (DDS DRUG DELIVERY SERVICES GES) 15 May 1996 (1996-05-15) page 1, line 1 - line 23 page 6, line 41 - page 8, line 17 claims 1-3,6,10,14-28; figures 13,14,17; examples ---	1-12, 14-16, 18-20
X	WO 98 07414 A (RES TRIANGLE PHARMACEUTICALS L) 26 February 1998 (1998-02-26) page 2, line 24 - page 3, line 16 page 4, line 10 - line 12 page 4, line 26 - page 7, line 2 claims 1-9; examples --- -/--	1-11,14, 15,18-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 1999	13/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Epskamp, S

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/US 99/11888

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 091 187 A (HAYNES DUNCAN H) 25 February 1992 (1992-02-25) cited in the application column 7, line 47 - line 63 column 10, line 1 - line 19 column 13, line 61 - column 15, line 52 column 16, line 19 - column 17, line 6 figure 1; examples 1,6,10	1-11, 16-20
X	US 5 100 591 A (CERFONTAINE PATRICK ET AL) 31 March 1992 (1992-03-31) column 1, line 61 - column 2, line 9 column 2, line 52 - column 3, line 26 column 3, line 56 - line 60 claims 1-9,14,15; examples 1,3	1-12, 18-20
A	US 5 663 198 A (REUL BERNHARD ET AL) 2 September 1997 (1997-09-02) column 2, line 17 - line 63 column 3, line 26 - line 36 claims 1,2,10; examples 1,5	1,12,13

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 99/ 11888

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1 and 2 relate to a product defined by reference to a desirable characteristic or property of components of the product, namely "the ratio of active substance to surface modifier and thermoprotective agent (is) selected to provide particle size stability during and after terminal steam sterilization, provided that the particle size is not more than an about two-fold increase in the weighted mean particle size of the particulate aqueous suspension subsequent to terminal steam sterilization." (claims 1 and 2) and "devoid of surfactants that require elevation of their cloud point temperature by addition of a cloud point modifier for the further stabilization and substantially devoid of surfacant additives which cause destabilization of the formulation." (claim 2).
The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the products as exemplified in Examples 1, 8 and 9. For claim 2 it has been taken into account that applicant apparently wishes to exclude the following agents (see p.2 l.27 - p.3 l.2 and p.4 l.1 - l.5): polyethylene glycols, polyvinyl alcohol, polyvinylpyrrolidone.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter . nal Application No
PCT/US 99/11888

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4440337 A	15-05-1996	AU 3982795 A	06-06-1996
		CA 2205046 A	23-05-1996
		CN 1172428 A	04-02-1998
		CZ 9701426 A	15-10-1997
		DE 19581305 D	05-11-1998
		WO 9614830 A	23-05-1996
		EP 0790821 A	27-08-1997
		FI 971986 A	08-07-1997
		HU 77526 A	28-05-1998
		JP 10508614 T	25-08-1998
		NO 972142 A	26-06-1997
		PL 320085 A	15-09-1997
		SK 58497 A	05-11-1997
		US 5858410 A	12-01-1999
WO 9807414 A	26-02-1998	AU 2587197 A	06-03-1998
		EP 0925061 A	30-06-1999
		NO 990790 A	19-04-1999
		US 5922355 A	13-07-1999
US 5091187 A	25-02-1992	US 5091188 A	25-02-1992
		AT 181234 T	15-07-1999
		AU 7852891 A	11-11-1991
		CA 2078990 A	27-10-1991
		DE 69131349 D	22-07-1999
		EP 0533690 A	31-03-1993
		IN 173056 A	05-02-1994
		MX 25532 A	01-10-1993
		RU 2100030 C	27-12-1997
		WO 9116068 A	31-10-1991
		US RE35338 E	24-09-1996
		US 5246707 A	21-09-1993
US 5100591 A	31-03-1992	FR 2651680 A	15-03-1991
		AT 93385 T	15-09-1993
		CA 2025298 A	15-03-1991
		DE 69002905 D	30-09-1993
		DE 69002905 T	23-12-1993
		DK 418153 T	12-08-1996
		EP 0418153 A	20-03-1991
		ES 2060107 T	16-11-1994
		GR 3020068 T	31-08-1996
		JP 2831455 B	02-12-1998
		JP 3169808 A	23-07-1991
		MX 9203803 A	01-08-1992
US 5663198 A	02-09-1997	DE 4323636 A	19-01-1995
		CA 2128034 A	16-01-1995
		EP 0634166 A	18-01-1995
		JP 7053353 A	28-02-1995